Universitat de Lleida

INFLUENCE OF PRODUCTIVE VARIETY AND THE DIETARY CRUDE PROTEIN ON FRACTIONAL PROTEIN SYNTHESIS RATE IN GROWING PIGS

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Abbreviations

AA: Amino acids

ADF: Acid- detergent fibre

ADG: Average daily gain

AEE: Acid hydrolyzed ether extract

AOAC: Association of official analytical chemists

BW: Body weight

CEP: Centre d'Estudis Porcins

CP: Crude protein

D: Dietary Crude Protein level

DM: Dry matter

F2: Pietrain piglets

FCR: Feed conversion ratio

FEDNA: Fundación española para el desarrollo de la nutrición animal

FM: Fresh matter

FSR: Fractional synthesis rate

G: Genotype/ productive variety

HP: High protein diet

IM: Intramuscular administration

ld: *Longissimus dorsi*

LP: Low protein diet

MC: Metabolic cage

MPE: Molar percent excess

MRM: Multiple Reaction Monitoring method

N: Nitrogen

NDF: Neutral-detergent fibre

OM: Organic matter

Phe: Phenylalanine

RD: Real Decreto

SID: Standardized ileal digestibility

TiO_2: Titanium dioxide

VFA: Volatile fatty acid

1. Abstract

The general scope is to analyse the effect of genotype and/or productive variety together with dietary protein supply on real animal requirements to improve further precision feeding and productive efficiency. To this end, a trial was conducted with 16 pigs of two different varieties, Pietrain and Duroc of 30.5 + 3.61 and 25.2 + 2.56 kg of BW respectively, fed with two diets with different protein content (LP 15%, HP 17%). Performance, digestive efficiency and fractional synthesis rate (FSR) using the flooding dose were analysed.

The Pietrain pigs presented a higher feed intake ($P < 0.05$) compared to the Duroc, although they didn't show growth differences. The protein content didn't influence the feed intake (dry and organic matter), except in the intake of crude protein (CP), with average daily gain being higher in the animals fed the LP diet than those with the HP ($P < 0.05$), especially notable in the Pietrain variety, whose growth (kg/day) was (0.33 ± 0.08 HP vs. 0.53 ± 0.08 LP), with respect to Duroc (0.38 ± 0.04 HP vs. 0.36 ± 0.05 LP). Only the apparent dry matter (DM) digestibility was higher in Duroc pigs; whereas the digestibility of the DM and the real one of the CP were higher in pigs fed the HP diet. The FSR was not affected either by the productive variety or by the level of CP in the diet; although the values tended to be higher for the Duroc and in the animals fed the HP diet.

Keywords: Performance, digestibility, fractional synthesis rate (FSR)

2. Resumen

El alcance general es analizar el efecto del genotipo y/ o la variedad productiva junto con el suministro de proteína dietética en los requerimientos reales de los animales, para mejorar la alimentación de precisión y la eficiencia productiva. Para ello, se realizó un ensayo con 16 cerdos de dos variedades diferentes, Pietrain y Duroc de 30.5 ± 3.61 y 25.2 ± 2.56 kg de peso corporal respectivamente, alimentados con dos dietas con diferente contenido de proteína (LP 15%, HP 17%). Se analizaron el rendimiento productivo, la eficiencia digestiva y la tasa de síntesis fraccional (FSR) utilizando la dosis de inundación.

Los cerdos Pietrain presentaron un mayor consumo de alimento ($P < 0.05$) respecto de los Duroc, aunque no presentaron diferencias en el crecimiento. El contenido proteico no influyó en el consumo de alimento (materia seca y orgánica), excepto en la ingestión de proteína bruta (CP), siendo mayor la ganancia media diaria (ADG) en los animales alimentados con dieta LP respecto de la HP ($P < 0.05$), especialmente notable en la variedad Pietrain, con un crecimiento (kg/día) de (0.33 ± 0.08 HP vs. 0.53 ± 0.08 LP), respecto de los Duroc (0.38 ± 0.04 HP vs. 0.36 ± 0.05 LP). Solamente la digestibilidad aparente de la materia seca (DM) fue mayor en los cerdos Duroc; mientras que la digestibilidad de la DM y la real de la CP resultó ser mayor en los cerdos alimentados con la dieta HP. El FSR no se vio afectado ni por la variedad productiva ni por el nivel de CP en la dieta, aunque los valores tendieron a ser mayores para los Duroc y en los animales alimentados con dieta LP.

Palabras clave: Rendimiento productivo, digestibilidad, tasa de síntesis fraccional (FSR)

3. Resum

L'abast general és analitzar l'efecte del genotip i/ o la varietat productiva juntament amb l'administració de proteïna dietètica en els requeriments reals dels animals, per millorar l'alimentació de precisió i l'eficiència productiva. Per a això, es va realitzar un assaig amb 16 porcs de dues varietats diferents, Pietrain i Duroc de 30.5 ± 3.61 i 25.2 ± 2.56 kg de pes corporal respectivament, alimentats amb dues dietes amb diferent contingut de proteïna (LP 15%, HP 17%). Es van analitzar el rendiment productiu, l'eficiència digestiva i la taxa de síntesis fraccionada (FSR) usant la dosi d'inundació.

Els porcs Pietrain van presentar un major consum d'aliment ($P < 0.05$) respecte dels Duroc, encara que no van presentar diferències en el creixement. El contingut proteic no va influir en el consum d'aliment (matèria seca i orgànica), excepte en la ingestió de proteïna bruta (CP), sent major el guany mig diari (ADG) en els animals alimentats amb dieta LP respecte de la HP ($P < 0.05$), especialment notable en la varietat Pietrain, amb un creixement (kg/dia) de (0.33 ± 0.08 HP vs. 0.53 ± 0.08 LP), respecte dels Duroc (0.38 ± 0.04 HP vs. 0.36 ± 0.05 LP). Solament la digestibilitat aparent de la matèria seca (DM) va ser major en els porcs Duroc; mentre que la digestibilitat de la DM i la real de la CP va resultar ser major en els porcs alimentats amb la dieta HP. El FSR no es va veure afectat ni per la varietat productiva ni pel nivell de CP a la dieta, encara que els valors van tendir a ser més elevats en els Duroc i en els animals alimentats amb la dieta LP.

Paraules clau: Rendiment productiu, digestibilitat, taxa de síntesi fraccionada (FSR)

4. Introduction

The increase in the demand of animal's products expected in the coming decades due to the increment in world population and its purchasing power, could double by 2050 (FAO, 2007), being the poultry and pork the main contributors, as well as the ones that more environmental impact produce.

The concern of today's consumers towards a more efficient production by optimizing natural resources and decreasing environmental pollution, leads to the development of different strategies to obtain animal products in a more efficient and respectful way. Precision feeding consists in provide the necessary nutrients to the animal according to their needs, in all genotypes or productive varieties, physiological stages, individually or in group. Refining requirements and supplies promotes a more productive efficiency and reducing the environmental impact, and then contributing to a more sustainable production.

Differences in protein metabolism obtained between different genotypes and/or productive variety of pigs (i.e. castrated Duroc vs. entire Pietrain, Seradj et al. 2017), suggested that genetic selection pressure exerted over the different pigs lines to get different organoleptic and/or productive characteristics, would also alter its real requirements and then it would be necessary to re-adjust the nutrient requirement and supply.

Our objective in the present assay is to analyze the influence of the genotype/productive variety and dietary crude protein (CP), on digestive efficiency and protein deposition. Real and apparent protein digestibility, absorption and utilization levels will be also analysed, which included the fractional (FSR) synthesis rate in two tissues, muscle (*Longissimus dorsi)* and liver.

5. Experimental design

For this objective, growing pigs between 25-30 kg BW were employed, bellowing to two genetic varieties: entire male pigs F_2 [Duroc × Landrace] dams × Pietrain sires, which is a representative of a commercial hybrid used for producing lean meat, this genotype is characterized by high growth and protein retention rates. Pigs are fattened until 90 kg of body weight (BW), using diets with high content of energy and high quality protein.

Pure castrated Duroc is a fatty variety employed to produce heavy pigs, with a sacrifice weight exceeding 120 kg BW and focused to obtain specific pieces, as dry-cure hams or loin. Males of the latter, are castrated before 7 days of life to avoid the sexual odour and flavour and to improve fat infiltration into the meat pieces, which improve organoleptic quality and its economic valuation (Cameron et al., 2000).

Requirements during the growing period depend directly from tissue retention, mostly protein and fat. In the former protein retention is mostly determined by the equilibrium of two processes, synthesis and degradation rate at different levels and tissues. The aim in the present approach is try to elucidate the effect of two factors, i) genetic/productive varieties and ii) dietary protein availability in protein degradation, absorption and synthesis in different tissues, in our case liver and muscle.

Crude protein digestibility was determined by the difference between ingestion and excretion (apparent digestibility), while true one was estimated from ileal samples obtained after animals sacrifice, using an external flow marker (Titanium Dioxide) supplied in the food during the whole experimental period.

Fractional (FSR) synthesis rate was determined using the "Flooding dose Technique", initially proposed by Garlick et al. (1980); and later developed by Rivera-Ferre et al. (2005) and Abecia *et al.* (2011). This allows calculating FSR in vivo in a wide range of tissues, characterised by a high or medium protein turnover rate. Specifically, by an intravenous administration of a massive dose of a labelled amino acid, to flood the precursor pools and reach the isotope equilibrium in the considered pools, and to maintain such equilibrium during the analysed period (Henshaw et al., 1971). Tissue free amino acids as the precursor pool rise its isotopic enrichment, which reaches levels close to that in plasma followed by a slow but linear decline (Garlick et al., 1980), because of its incorporation in muscle protein. Measurements of the specific label of free and protein-bound amino acid in tissues, enables to determine the rate of protein synthesis during a period of time after the injection.

And finally, a liquid chromatography-tandem mass spectrometry, which is a relatively pioneer technique, was used to trace the kinetics of incorporation of the labelled amino acid into the precursor pools and newly synthesized proteins. This is possible due to the sensitivity of this sophisticate method to detect very low isotopic enrichments. The advantage of this method, unlike the gas chromatography, which has been the method of

choice up until recently, is that enzymatic conversion and derivatization of L-phenylalanine aren't necessary to improve its volatility, thermal stability and sensitivity in the detection. Thus makes the sample preparation simpler, avoiding potential errors (Wilkerling et al., 2012).

6. Materials and Methods

All procedures were carried out under Project License 9490;22-06-2017 and approved by the in-house Ethics Committee for Animal Experiments of Departament de Territori i Sostenibilitat, Direcció General de Polítiques Ambientals i Medi Natural from the Generalitat de Catalunya. The care and use of animals were in accordance with the Spanish Policy for Animal Protection RD 53/2013, which meets the European Union Directive 2010/63 on the protection of animals used for experimental purposes.

6.1. *Housing, Animals and Diets*

The study was conducted at the Centre d'Estudis Porcins de Catalunya, located in Torrelameu (CEP; Lleida, Spain).

Sixteen male piglets from 2 different genotypes (Pietrain and Duroc) were purchased from Selección Batallé®, Girona, Spain. The Pietrain (F2) piglets (8 piglets) were entire progeny of [F1: Duroc × Landrace] dams × Pietrain sires, whereas the Duroc (D) piglets (8) were castrated purebreds.

Animals were divided, from their origin, in 4 batches of different age and selected by body weights (23.5 ± 1.91; 21.1 ± 3.33; 15.3 ± 2.33 and 11.7 ± 1.04 kg BW), exposed in Figure 2 and 3 (Annexes), in a way that each batch contained 2 piglets of each genotype (2 F2, 2 D).

Once in the CEP, animals were identified and each batch (4 piglets) were allocated in separated pens, and fed with a commercial concentrate as in their origin farm.

The experiment started mid-May using the first batch. The four piglets were distributed in two different pens (2 piglets: one for each genotype) and feed was restricted to 1 kg (FM basis), offered in two equal meals (500 g) at 08:00 and 16:00 hours. The experimental diets (Table 1 and 2) were formulated to have the same energy content but with two levels of CP content (High [17%] or Low [15%]) for 3 days (adaptation to the diet).

In both diets (HP and LP) Essential Amino Acids (EAA) profiles and standardized ileal digestible (SID) content were maintained by including pure synthetic AA in the formulation process.

Afterwards, animals were confined in metabolic cages (MC; 200 x 104 x 71 cm) for seven days, (1 to 4 d for adaptation and 5 to 7 d for experimental sampling). MCs were disposed in the same room, distributed in parallel under identical controlled environmental parameters. Daily recorded temperatures are represented in Figure 4 (Annexes).

Once the animals of the initial batch began their sampling period in MCs, the sequent batch of piglets, once they reached to convenient weight (≈ 24 kg; considering ADG of 0.6 kg/d) started with the experimental period; the protocol was repeated with all the four batches.

Table 3 Ingredients (g/ kg DM) of the experimental diets, differing in CP content (high, HP vs. low, LP) for piglets of 10 weeks of age

Ingredients (g/ kg DM)	Experimental Diets	
	LP	HP
Maize/ Corn	294.8	246.5
Barley	290.0	287.8
Wheat	200.0	200.0
Soybean meal*	137.6	195.0
Beet pulp, dehydrated[a]	30.0	30.0
Calcium carbonate	13.4	13.2
Mono Calcium phosphate	9.4	8.8
Soybean oil	9.0	6.3
L-Valine	6.8	8.0
Sodium chloride	4.6	4.6
L-lysine HCL	4.2	2.4
Vitamin- Mineral Premix[b]	4.0	4.0
L-Threonine	1.6	0.8
DL- Methionine	1.0	0.5
L-Tryptophan	0.3	2.0

*Soybean Meal as the sole protein source of the diet was partly defatted (contained 7.8 % of Etheric Extract) with low residual trypsin inhibitor activity and supplied by Terres Inovia (France). Its inclusion was mainly due to requested collaboration with the WP1 of F-a-G.

[a]Sugar beet pulp was used (30 g/ kg) to cover the fibre needs mainly in initial diets.

[b]The vitamin and mineral premix for pigs between 9 and 15 weeks of age contained (per kg of complete diet): 2,4 mg of vitamin A; 0,02 mg of vitamin D3; 40 mg of α-tocopherol; 2.4 × 10−2 mg of vitamin B12; 0.8 mg of vitamin B1; 1.6 mg of vitamin B6; 4 mg of vitamin B2; 1.2 mg of vitamin K3; 16 mg of nicotinicacid; 8 mg of pantothenic acid; 280 mg of choline chloride; 0.08 mg of biotin; 0.4 mg of folic acid; 72 mg of Fe (FeCO3); 0.32 mg of I (KI); 0.16 mg of Co (CoSO4·7H2O); 128 mg of Cu (CuSO4·5H2O); 23.8 mg of Mn (MnO); 80 mg of Zn (ZnO); 0.24 mg of Se (Na2O3Se); 0.264 mg of citric acid; 600 FYT 6-phytase;2000 BGU of endo-(1,4)-ß-glucanase; 4800 FXU of endo-(1,4)-ß-xylanase; 0.264 mg of ethoxyquin.

LP, Low level of Crude Protein content used in diet (regarding to recommendation of FEDNA for pigs of that age) and HP, High level of Crude protein used in diet (regarding to recommendation of FEDNA for pigs of that age).

In addition, diets were fortified to meet vitamin and mineral requirements (FEDNA, 20013) and phytase was added to improve the digestibility of phosphorus.

Table 4 Chemical composition (g/ Kg DM) together with estimated values of Energy in experimental diets, differing in CP content (high, HP vs. low, LP) for pigs of 11 weeks of age

Nutrients (g/ kg FM)	**Experimental Diets**	
	LP	**HP**
Dry matter (g/kg FM)	866.7	867.9
Crude Protein	147.2	166.9
Ether Extract	29.8	26.6
Crude Fibre	33.9	35.1
Ash	54.3	56.4
Starch	462.1	430.3
Total Sugar	30.2	34.7
NDF	134.1	133.8
ADF	42.6	44.0
ADL	7.5	7.5
LYS, SID	9.0	9.0
THR, SID	5.8	5.8
MET, SID	3.0	2.8
MET + CYS, SID	5.3	5.4
TRP, SID	1.7	1.7
ILE, SID	5.0	6.0
VAL, SID	5.8	6.8
LEU, SID	10.0	11.1
PHE, SID	6.0	7.2
HIS, SID	3.1	3.7
ME (kcal/kg)	3108.0	3094.0
NE (Kcal/ kg)	2353.0	2303.0

LP, Low level of Crude Protein content used in diet (regarding to recommendation of FEDNA for pigs of that age) and HP, High level of Crude protein used in diet (regarding to recommendation of FEDNA for pigs of that age).

7. Animal's handling and experimental management.

7.1. *Surgery, jugular catheterization*

Twenty four hours before infusion trial (day 6) animals were sedated with intramuscular administration (IM) of Stresnil(R) (ESTEVE, S.A. Barcelona; Spain), and then anesthetized using IM injection of Domtor(R) (Orion Corporation, Espoo; Finland) and Ketamidor(R) (Karizoo laboratories S.A., Caldes de Montbui; Spain) in the MCs and transferred to the operating room, where they were subjected to catheterization surgery at their right jugular vein. The incision area was cleaned, shaved and disinfected with povidone- iodine, and the right external jugular vein was located using a triangulation technique described by Flournoy & Mani (2009). A dual catheter set (Certofix(R), Braun Medical S.A., Rubí; Spain) was placed into the external jugular vein of the animal, following guide-wire-assisted percutaneous cannulation method for minimal invasive vascular access,(Flournoy & Mani 2009). Once the catheter was placed was flushed with approximately 0.5 – 1 mL of heparinised saline (Heparina Hospira(R) 1%). Finally, to provide more security the neck and part of the thorax was covered with a wide adhesive tape (20 cm width). Afterwards the animal was transferred to the MC still anesthetized and monitored about 2 hours until full recovery.

7.2. *Experimental management*

Throughout the experimental period feed intake and water consumption was recorded (day 1 to d7); feed intake by difference between offer and ort, and water consumption recorded individually using Water-flow counters.

Blood samples (≈ 7 ml) were taken daily (d3 to d7) through the left jugular vein, using BD Vacutainer® with K_2EDTA as anti-coagulant. Blood samples were immediately centrifuged (10 min - 600g), and plasma stored in cryogenic tubes (2 mL) at – 20 ºC. Daily urine volume was also measured and samples were collected in a plastic jar under H_2SO_4 (100 mL 10 %) beneath each cage, and stored at – 20 ºC. Apparent total tract digestibility was estimated using Titanium Dioxide (TiO_2) as an external marker of digestibility, fecal spot sample (≈ 70 g) was obtained (d5 to 7) by rectal stimulation. Fractional protein synthesis rate (FSR) in tissues was determined following the flooding dose procedure proposed initially by Garlick, et al. (1980). Phenylalanine (5–10 times the amount of the pig's free phenylalanine pool) was prepared dissolving 2.89 g of phenylalanine (containing 15% labelled L-Phenyl-D5-alanine ($^2H_5$-Phe)) in 100 mL of sterile saline. Labelled AA was from Cambridge Isotope Laboratories (CIL

Laboratories) Andover (USA); whereas, the unlabelled L-Phenylalanine was purchased from Sigma-Aldrich, Steinheim (Germany). Phenylalanine solution was infused for 10 min by means of 10 aseptic syringes of 10 mL. Before phenylalanine infusion a blood sample (≈ 7 ml) was taken (as was previously described) to determine the natural enrichment; immediately after infusion series of (≈ 6mL) blood samples were taken at 12, 15, 20, 25, 30, and 40 min to define the enrichment curve of the free phenylalanine pool in plasma and tissues. After blood samplings, heparinised saline was flushed by the catheter to ensure patency of the catheter and to maintain blood volume. At the end of the sampling period, the animals were immediately euthanized with a lethal IV dose of 10-15 mL of Euthasol® (ESTEVE S.A., Oudewater Netherlands). Straightaway after sacrifice, the body was weighed and ventral side was opened, a sample of urine was taken by means of a syringe directly from the bladder. The whole gastrointestinal tract was ligated, excised, weighed and sampled. The ileum digesta was subsampled (5 g) to measure the standardized ileal digestibility (SID) of Amino Acids (AA) content using Titanium Oxide (TiO2) as an external marker of digestibility inside the experimental diets.

To determine FSR, (2-3 g) of the muscle *Longissimus dorsi* (ld) from the left half of the corpse and liver were also sampled, and samples were collected in falcon tubes of 15 mL immediately frozen on dry ice, once transported to the laboratory, the samples were kept frozen at – 80 °C until further analyses.

8. Chemical analyses

Feed, digesta (ileum, cecum, mid colon and distal colon) and fecal samples were analyzed in duplicate following recommendations of the AOAC (2005) for DM (ref. 934.01), Ash (Incineration at 550ºC) and N content (Kjeldahl method), starch (polarimetry), acid hydrolyzed ether extract (AEE; Soxhlet method), NDF and ADF contents (sequential procedure), following Van Soest et al. (1991). Neutral-detergent fibre was assayed with a heat-stable amylase and expressed exclusive of residual ash (aNDFom). Hemicellulose content was calculated by difference between aNDFom and ADFom.

The amino acids content of the ileal digesta and offered feed (LP and HP) were determined by hydrolysis of samples (50 mg), incubated under N stream in 5 mL 6N HCl for 12 h at 110°C (Colgrave et al. 2008) and centrifuged at 3000g at 4ºC for 30

min. Quantitation of individual AA was performed using a method described by Guo et al. (2013) with the following modifications. An ultra-high-performance liquid chromatography acquity system (Waters, Milford, MA, USA) holding a BEH amide column (2.1 · 150 mm; 1.7 mm) was used. Solvent A was 10 mM ammonium formate in water with 0.15% formic acid; solvent B was ammonium formate-saturated acetonitrile with 0.15% formic acid. The gradient included five steps. Initial conditions were 15% A and 85% B maintained for 3 min at 0.5 mL/min. Then, from 15% to 20% A in 3 min; from 20% to 24% A in 1.5 min; from 24% to 60% A at 0.6 mL/min in 1.5 min and maintained for 3 min. Then, initial conditions were regained in 2 min. Weak and strong washing solvents were 80% acetonitrile and 20% acetonitrile, respectively. Samples were filtered through a 0.20-mm hydrophilic PTFE membrane before injection. The injection volume was 5 mL. Quantisation of amino acids in the hydrolysate was performed by using a Multiple Reaction Monitoring method (MRM) in a Waters TQD mass spectrometer (Micromass MS Technologies, Manchester, UK). Briefly, the system was equipped with an ESI source operated in positive ion mode. The parameters in the source were set as in Guo et al. (2013). Moreover, their MRM transitions were tested successfully in our conditions for phenylalanine, leucine, isoleucine, methionine, valine, proline, tyrosine, alanine, threonine, glycine, glutamic acid, serine, aspartic acid, histidine, arginine, lysine and cistine. Cone voltage and collision energy were optimised for each individual amino acid. Calibration curves were constructed from a commercial amino acid standard mixture (Ref.: AAS19, Sigma-Aldrich, St Louis, MO, USA) and diluted to a series of appropriate concentrations with water/acetonitrile (20/80 v/ v). Triptophan concentration was not determined due to its degradation under the conditions described as above (Fountoulakis and Lahm 1998). The results were processed using QuanLynx software (MassLinx, Waters Corporation, Milford, MA, USA).

L-Enrichment of Phenyl-D5-alanine] was performed as follow:

(i) Plasma samples. Plasma samples were prepared as described in Piraud et al. (2005). Briefly, 200 µL of plasma were mixed with 800 µL of methanol, vortexed for 2 min and preserved at room temperature for 10 minutes. Then were centrifuged for 5 min at 17500 g, and an aliquot of 50 µL was obtained from the supernatant.

(ii) Liver and muscle samples. A sample of 300 mg of tissue was freeze-dried and homogenized by mixing with sand using a glass stirring rod. Free amino acids were extracted twice following the method used by Qin et al. (2015). Samples were incubated

twice in 500 µL water and 500 µL methanol, at 4ºC for 30 min (shaking it continuously), and then centrifuged at 10,000 x g for 10 min. The supernatants were removed, mixed and an aliquot of 100 µL were recovered and stored. The remaining pellet was used to determine protein bounded amino acids using the method described by Colgrave et al. (2008). It was submitted to hydrolysis by adding 5 mL of 6N HCl containing 0.02% phenol, sealing the tubes with N_2 and incubate it for 24 h at 110ºC. After hydrolysis, tubes were cooled and centrifuged at 3,000 g for 30 min. 50 µL of the supernatant was recovered and 100 µL of distilled water was added. The aliquots of plasma, liver and muscles were evaporated and rediluted in 500 µL of water/acetonitrile (15/85 v/v). Then the mixture was vortexed, filtered through a 0.20 µm hydrophilic PTFE membrane and injected in Ultra-High-Performance Liquid Chromatography (UHPLC) Acquity system (Waters, Milford, MA), equipped with a BEH Amide column (2.1 x 150 mm; 1.7 µm). Solvent A was 10mM ammonium formate in water with 0.15% formic acid; while the solvent B was ammonium formate-saturated acetonitrile with 0.15% formic acid. The gradient included five steps. Initial conditions were 15% A and 85% B maintained for 3 min at 0.5 mL/min. Finally, from 15% to 20% A in 3 min; from 20% to 24% A in 1.5 min; from 24% to 60% A at 0.6 mL/min in 1.5 min and maintained for 3 min. Then initial conditions were regained in 2 min. Weak and strong washing solvents were 80% acetonitrile and 20% acetonitrile, respectively. The injection volume was 5µL. Quantification of amino acids was performed by using a Multiple Reaction Monitoring method (MRM) in a Waters TQD mass spectrometer (Micromass MS Technologies, Manchester, UK). Briefly, the system was equipped with an ESI source operated in positive ion mode. The parameters in the source were set as in Guo *et al.* (2013). Phe and [phenyl-$^2H_5$]Phe MRM transitions were determined in our conditions and cone voltage and collision energy were optimized for each individual amino acid (Table 1). The absence of crossed signal between both amino acids was checked.

Table 3 Precursor/Product Ion pairs and parameters for Multiple Reaction Monitoring (MRM) of Phe and [phenyl-$^2H_5$]Phe

Fatty acid	Retention time	$[M-H]^-$ *(m/z)*	MRM Transition	Cone voltage	Collision energy
PHE	2.91	166.10	166.10 >103.00	20.0	22.0
			166.10 > 120.10	20.0	14.0
[phenyl-$^2H_5$]Phe	2.89	171.22	171.22 > 106.14	20.0	25.0
			171.22 > 125.26	20.0	15.0

Ammonia-N concentration was determined (Chaney and Marbach, 1962). Flow marker concentration (TiO_2) in feed, fecal and digesta samples was analysed following Leone (1973).

9. Calculations and Statistical analyses

Digestibility of components was calculated from flow marker concentration in the feed and digesta samples as follows:

$$y = [1 - ([TiO_{2\ feed}] / [TiO_{2\ digestive\ segment}]) \times (Z_{digestive\ segment} / Z_{feed})]$$

where *y* represent the digestibility of a nutrient at a certain segment (i.e. fecal and digesta samples collected from the ileum, cecum, mid-colon and distal colon), $[TiO_{2\ feed}]$ and $[TiO_{2\ digestive\ segment}]$ represent the concentration of TiO_2 in feed and that certain segment, respectively; and $Z_{digestive\ segment}$ and Z_{feed} are the nutrient concentrations (g/kg) in that specific intestinal tract segment and in the diet, respectively.

Fractional synthesis rates of protein (FSR) in organs or tissues, which is defined as the percentage of tissue protein synthesised per day (%/ day), is calculated from the following equation:

$$FSR\ (\%/\ day) = (MPE_{bound} \times 100) / (MPE_{free} \times t)$$

where $\mathbf{MPE_{bound}}$ is the labelled phenylalanine isotopic enrichment (%) in the protein of the target organ or tissue (assuming that no $^2H_5$Phenylalanine in the background sample does exist)**.** $\mathbf{MPE_{free}}$ is the average of the isotopic enrichment (%) in the free phenylalanine pool in the tissue; considering that the free-pool enrichment in plasma over time was described linearly as: $MPE_{free} = at + b$. And **t** is the time (days) of the tracer labelling (from the beginning of the infusion to the freezing of tissues).

The results were analysed by a software package (SAS Institute) and examined by two-way ANOVA, in which the protein level (Low Protein (LP) 15 % CP vs. High Protein (HP) 17% CP) and pig breed (Duroc vs. Pietrain) were considered the main effects, also considering the interaction between both. Statistical differences were considered significant when $P < 0.05$.

10. Results and Discussion

10.1. *Ingestion and Performance*

The data obtained from the feed intake (Table 4) reveal that significant differences between productive varieties/ genotypes does exist, being the consumption (DM based) higher in the Pietrain's than in Duroc ($P < 0.05$), and then, intake of organic matter (OM) and crude protein (CP) followed the same tendency. Diet did not alter, neither DM nor OM intake although, those animals feed HP diets ingest a higher level of CP ($P < 0.05$).

Despite the differences recorded on intake, differences were not reflected on growth, which not showed significant differences between productive varieties. Average daily gain (ADG) was higher in those animals fed LP diets ($P < 0.05$), although this factor interacts between productive variety (i.e. genotype) and CP on Diet, Pietrain variety growth faster in those fed LP (0.33 ± 0.08 HP vs. 0.53 ± 0.08 LP), although dietary difference between Duroc were not noticeable (0.38 ± 0.04 HP vs. 0.36 ± 0.05 LP). Whereas feed conversion ratio (FCR) was higher in HP animals ($P < 0.05$), being more evident in Pietrain (2.95 ± 0.214 HP vs. 1.84 ± 0.25) than in Duroc (2.32 ± 0.21 vs. 2.33 ± 0.21).

Table 4 Feed intake (g/ d) of dry matter (DM), organic matter (OM) and crude protein (CP), and performance of 24 pigs, 12 entire Pietrain and 12 castrated Duroc fed two levels of dietary CP

	Productive Type		Protein Level			Signification		
	Duroc	**Pietrain**	**HP**	**LP**	**SEM**	**G[1]**	**D[2]**	**DxG**
Intake								
DM	778,8	854,3	821,9	811,1	20,49	0,02	0,71	0,35
OM	726,6	797,1	764,4	759,2	19,10	0,02	0,85	0,34
CP	135,0	147,7	152,9	129,8	3,63	0,03	0,001	0,46
Growth and Feed conversion ratio								
ADG[3]	0,37	0,43	0,36	0,45	0,02	0,09	0,02	0,005
FCR[4]	2,33	2,40	2,64	2,09	0,53	0.75	0.03	0.03

[1]G: Productive variety/ genotype; [2]D: Dietary Crude Protein level; [3]ADG: Average daily gain (kg/ d); [4]FCR: Feed conversion ratio.

The results of both DM intake and ADG obtained in this trial are lower than those obtained by Rivera-Ferre et al., (2005), whose also worked with different productive varieties of pig (Iberian and Landrace) of similar weights to ours, and with two dietary protein levels, although lower than those used in the current trial (12 vs. 16 % CP). Performance for Iberian and Landrace pigs was 455 ± 37.45 and 675 ± 53.75 g/ d respectively. Considering that the diets were adjusted and balanced in AA for pigs between 20 and 60 kg of BW (FEDNA, 2013), differences on ADG are difficult to justify.

On the other hand, the Pietrain type is considered a meat pig variety (MAPA, 2018), it has been harshly selected and improved to increase its performance. In fact, it's frequently used as a terminal sire in cross-breed pigs with the aim to transmit to its fattening offspring its leanness and musculature. This great specialization has led to a high intake capability but a low capacity for adaptation to limiting conditions, as in our case could be an excessive content of protein.

It has been demonstrated that growth rate improves as the dietary CP content increases to levelled off at one specific level (Campbell et al. (1984), Carpenter et al. (2004)), after that animal's need to eliminate the excess of N expending energy. Therefore, ADG and protein accretion may decreased linearly as dietary CP increased (Chen *et al.*,

1999). That would explain the decrease in growth detected within Pietrain's varieties increasing the level of CP supply.

However, Duroc it's a variety with a lower genetic selection pressure than the Piertain and moreover its selection has been focused mainly to, i) maintain its rusticity and ii) to increase its fatty infiltration to elevates organoleptic characteristics and so its commercial category (ANPS, 2018). Such fact may allow this animal to adapt to different climatic conditions and /or quality feeds. This reason may justify the null effect of protein concentration on Duroc growth.

10.2. *Digestibility*

Apparent digestibility account for the total nutrients digested in the low gut plus the material fermented and absorbed in the hindgut (mostly VFA, ammonia and minerals), whereas that true digestibility corresponds only to the nutrients digested in the former part, consequently apparent should be higher than true digestibility (Table 5). However, microbial yield in the fermentation compartment (caecum plus colon in the hindgut tract) using endogenous N source (mostly from plasma urea, Belenguer *et al.*, 2005) may induce a net appearance of protein (also DM) in to the hindgut and consequently a significant reduction of total (apparent) digestibility (Van Soest, 1982). That fact may explain the low values of the apparent CP-digestibility in relation to the true digestibility of this component reported in table 5. In this sense Stein *et al.*, (2007) also suggest that differences in the endogenous contribution to low gut-AA flow may alter values of the true digestibility mostly in mono-gastric animals.

Most digestibility values tend to be higher in Duroc pigs, although only DM apparent digestibility reached statistical significance, being ($P < 0.05$). On the other hand, animals fed with HP level showed a larger nutrient (i.e. DM and CP) true digestibility than those fed LP ($P < 0.05$).

Diets with higher CP content result in higher digestibility of nutrients, including amino acids, as detailed by Rérat et al., 1988. A greater contribution of amino acids to the animal by these diets is the reason for this fact, leading to an improvement of the digestive processes. Moreover, the fact that the digestibility was higher in Duroc pigs could be due to its greater rusticity and ability to adapt to extreme circumstances, unlike the Pietrain, as discussed above; since under normal conditions, the selected animals for the production of lean meat have better digestibility (Abércio et al., 2012).

Table 5 Apparent and True digestibility (%) of DM, OM and CP registered in two productive types/genotypes and feed two levels of dietary CP.

	Productive Type		Protein Level			Signification		
	Duroc	**Pietrain**	**HP**	**LP**	**SEM**	**G[1]**	**D[2]**	**DxG**
Apparent Digestibility								
DM	92,4	90,9	91,6	91,6	0,24	0,00	0,93	0,03
OM	71,8	70,6	71,7	70,8	1,01	0,43	0,53	0,82
CP	62,2	61,1	64,0	59,4	2,37	0,75	0,20	0,71
True Digestibility								
DM	94,0	92,6	94,5	92,1	0,71	0,18	0,04	0,41
OM	52,1	46,5	56,7	41,9	5,40	0,48	0,08	0,87
CP	59,1	60,3	68,1	51,3	1,90	0,68	0,004	0,30

[1]G: Productive variety/ genotype; [2]D: Dietary Crude Protein level.

Attending to the true amino acid (AA) digestibility we can

divide them in three groups (Table 6). The first one, composed by Methionine, with the highest digestibility coefficient, with 82.74 ± 5.03 % followed by the most abundant group, Cisteine, Isoleucine, Leucine, Lysine, Phenylalanine, Tyrosine and Valine; showing an average digestibility around 60 and 65 %. And finally, the amino acid with the lowest digestibility on average was Threonine, with 53 ± 6.69 %

In general, values of true amino acid digestibility are slightly lower than other values described in bibliography, in which average digestibility of essential AA is above 80 % (Furuya and Kaji, 1989; Leterme *et al.*, 1990; Liu *et al.*, 2016), also in growing pigs, however in relation to ranking obtained in true digestibility among essential AA, methionine is usually one of the amino acids showing greater digestibility (Furuya and Kaji, 1989; Liu *et al.*, 2016; Adeola and Ragland, 2016); whereas the threonine usually occupies the last positions in the rank (Furuya and Kaji, 1989; Leterme *et al.* 1990).

Disappearances of the essential AA throughout the low gut is leaded by two main processes, hydrolysis and transport though the intestinal mucosa. In relation to the former the hydrolysis of dietary protein to peptides a further to AA is a complex interaction between specific proteases and gut conditions, different AA are subjected to

different hydrolytic environment and it explain some of the important variation component recorded in the digestibility process (Blachier et al., 2013).

Table 6 True digestibility (%) of Essential Amino acids of DM, OM and CP registered in two productive types/genotypes and feed two levels of dietary CP

	Productive Type		Protein Level			Signification		
	Duroc	**Pietrain**	**HP**	**LP**	**SEM**	**G[1]**	**D[2]**	**DxG**
Real Digestibility								
Cisteine	59,57	62,44	63,34	58,67	5,89	0,74	0,59	0,12
Isoleucine	64,50	68,10	69,31	63,30	5,45	0,65	0,45	0,20
Leucine	64,92	59,81	67,28	57,45	5,99	0,56	0,27	0,54
Lysine	55,78	66,64	64,53	57,90	8,71	0,40	0,60	0,25
Metionine	82,65	82,83	85,60	79,87	5,03	0,98	0,44	0,66
Phenylalanine	64,20	69,26	71,95	61,50	6,16	0,57	0,26	0,26
Threonine	51,94	53,26	55,35	49,85	6,69	0,89	0,58	0,04
Tyrosine	59,79	60,71	67,63	52,87	6,23	0,92	0,12	0,17
Valine	63,65	68,14	69,50	62,29	5,37	0,57	0,36	0,19

[1]G: Productive variety/ genotype; [2]D: Dietary Crude Protein level.

In relation to the transport of AA across the intestinal border it is mediated though an active process where different transporters are involved, according to the existing literature (D'Mello, 2000; Bröer, 2008), the efficiency in which the different AA are bound with their specific transporter vary among AA and also transporters concentration in the intestinal mucosa border differ is affected by diet and it may be genetically controlled (Guixin et al., 1995; Ouweltjes *et al.*, 2018). In fact, it has been reported that pigs of native breeds have longer intestines and higher jejunal villi than the more developed breeds, fact that causes a longer retention time of intestinal content, increasing digestibility (Guixin et al., 1995). In this sense only threonine evidenced an interaction between both variables ($P < 0.05$) (Table 7). The digestibility of this AA was higher in animals fed with HP diet in Duroc variety, while in the Pietrain were the pigs fed with LP level.

Table 7 Interaction in True digestibility (%) between productive variety/genotype and CP content on diet of the Threonine

	Pietrain		Duroc			Signification		
	HP	**LP**	**HP**	**LP**	**SEM**	**G**	**D**	**DxG**
True Digestibility								
Threonine	44,6	61,9	66,1	37,8	6,77	0,89	0,58	**0,04**

10.3. *Fractional Synthesis Rate (FSR)*

The results obtained show that the FSR is higher in the liver than in the muscle, almost four times higher. In addition, values tend to be higher in Duroc than in Pietrain pigs, and in those animals fed the HP in relation to LP diet, although differences did not reach statistical significance.

Table 8 Fractional Synthesis Rate (%) in *Longissimus muscle* and liver in 24 pigs, 12 entire Pietrain and 12 castrated Duroc fed two levels of dietary CP

	Productive Type		Protein Level			Signification		
	Duroc	**Pietrain**	**HP**	**LP**	**SEM**	**G**	**D**	**DxG**
FSR								
Longissimus	17,0	14,9	16,8	15,2	1,68	0,41	0,51	0,55
Liver	65,0	57,3	63,0	59,4	4,87	0,29	0,61	0,79

Fractional Synthesis Rate is usually calculated in those tissues that contribute to body protein turnover in a major proportion. Muscle (i.e. *Longissimus dorsi*), which represents an important part of body composition (i.e. 40 % in standard pigs with 25 kg BW (Upton, 2008)), it's constituted mainly by protein, up to 20% of BW (Listrat *et al.*, 2016), and being the largest pool of amino acids present in the body (Seal and Parker, 2000). Liver also contributes significantly because this organ shows the highest metabolic activity into the body system (Munro, 1970).

According to our results, it is well described in the existing literature that some tissues as liver and also gut have much higher turnover rates than others as muscle, also its protein deposits (Wagenmakers, 1999; Burd et al., 2013). Differences between liver and

muscle were similar (using growing pigs and a similar protocol) publications (Rivera-Ferre et al., 2005, Wykes *et al.*, 1996, Edmunds & Buttery 1978 and Simon *et al.*, 1978); in which FSR on muscle and liver were (8.0 - 6.3; 46.8 – 44.3), (7.0; 42.0), (5.8; 36.9), and (8.1; 115) respectively. Although, magnitudes obtained in our trial, in values of both FSR in muscle and liver are higher than those mentioned above.

It is difficult to justify the existing differences, however if the experimental approaches are similar variations has to be related to: i) the specific infusion protocol and isotope equilibrium into the body pool under the flooding doses conditions or, ii) the analytical procedure.

In relation to the former, Figure 1 shows the evolution of isotopic enrichment (MPE %) of free phenylalanine in plasma over time, at 12, 15, 20, 25, 30 and 40 minutes after infusion (i.e. in the case of Duroc animals fed HP diet), together with the enrichment of registered in liver and muscle (*Longissimus dorsi*).

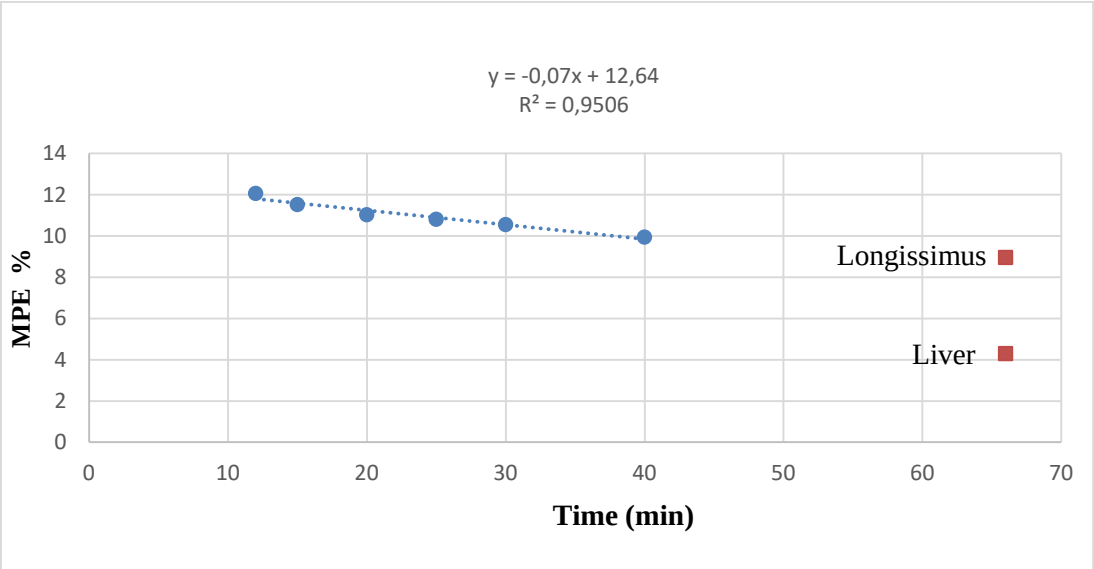

Figure 2 Plasma enrichment (molar percent excess (MPE)) of the free phenylalanine in plasma and in tissues after slaughtering, in a Duroc pig fed with high protein diet.

The graph shows the decay of the free AA enrichment as was described initially by Garlick et al., (1980) after the flooding dose in the plasma also the enrichment (MPE) in the analysed tissue. The isotope behaviour also the model was very similar that those proposed initially by Garlick et al., (1980) and later confirmed by Rivera-Ferre et al., (2005) so for instant differences in the both factors could be discarded.

In relation to the later in the present approach a liquid chromatography-mass spectrometry system was used instead of gas chromatography-mass spectrometry, our proposed methodology has higher selectivity and sensitivity and less sample handing (Wilkerling *et al.*, 2012), it may explain the quantitative variations reported. Unfortunately no previous experimental evidence using the analytical tandem does exist but the demonstrated efficiency with system support the methodology employed and the absolute values proposed.

No significant differences were detected in the FSR, between productive varieties/genotypes or in protein availability, however a trend observed from our animals agrees with previous results reported by Rivera-Ferre et al., (2005).

Effectively, Rivera-Ferre et al., (2005) obtained a higher FSR for both tissues in those animals fed with the HP diet, and in the Iberian pigs than in the Landrace. Comparing to the Iberian pigs, the productive variety Duroc has been submitted at lower selection pressure towards a higher production. Both Iberian and Duroc varieties showed growth rates lower than the Landrace variety, if in the former Rivera-Ferre et al., (2005) suggested that differences in ADG would be more related to the protein degradation rate in state of FSR in our case we can speculate in a similar growth control mechanism in Duroc varieties . Although that point need to be demonstrated experimentally.

Moreover, it has been studied that the deficiency or excess of certain AA (i.e. threonine) induces the decrease of the Fractional Synthesis Rate, mainly in skeletal muscle, jejunal mucosa and mucins (Wang *et al.*, 2007).

11. Conclusions

11.1. The lean variety Pietrain, due to its great genetic selection, is a breed that gets a higher consumption of fed than the Duroc breed, although due to its reduced ability to adapt to limiting conditions, in this case the excessive content of protein in the diet, caused a reduction in their ADG in those fed with the HP diets. However, the rusticity of the Duroc allowed them to adapt to the HP diet and obtained better growths than those fed with LP.

11.2. Digestibility tend to be higher in Duroc pigs given its rusticity/ adaptability; while a higher dietary CP content result in higher nutrient digestibility (i.e. AA).

11.3. True digestibility varied among the different essential amino acid and they ranked from 83 to 53 % showing higher digestibility values in methionine and lower in theornine.

11.4. FSR is higher in the liver than in the muscle, and values apparently were higher in Duroc than in Pietrain pigs and in those animals fed the HP in relation to LP diet.

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Fundación Española para el Desarrollo de la Nutrición Animal (FEDNA), 2016

13. Annexes

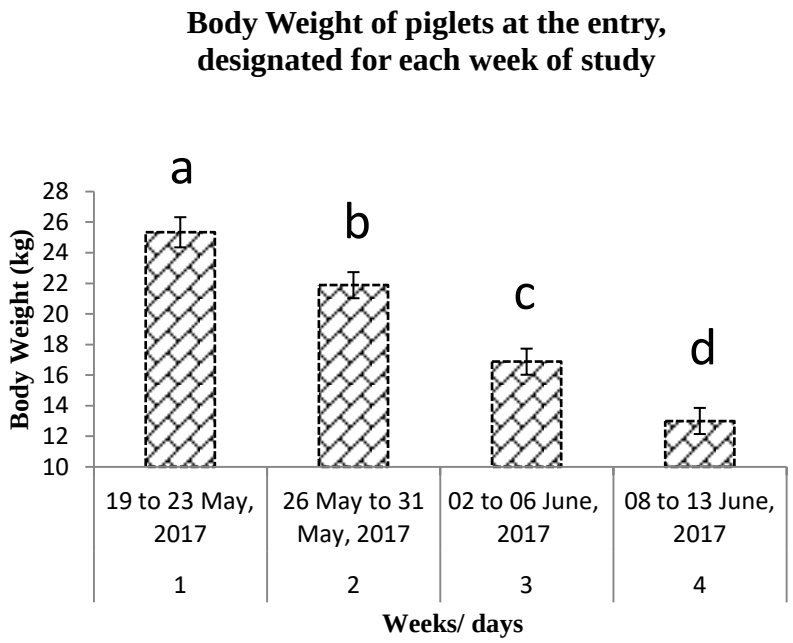

Figure 2 Body weights of piglets at the entry

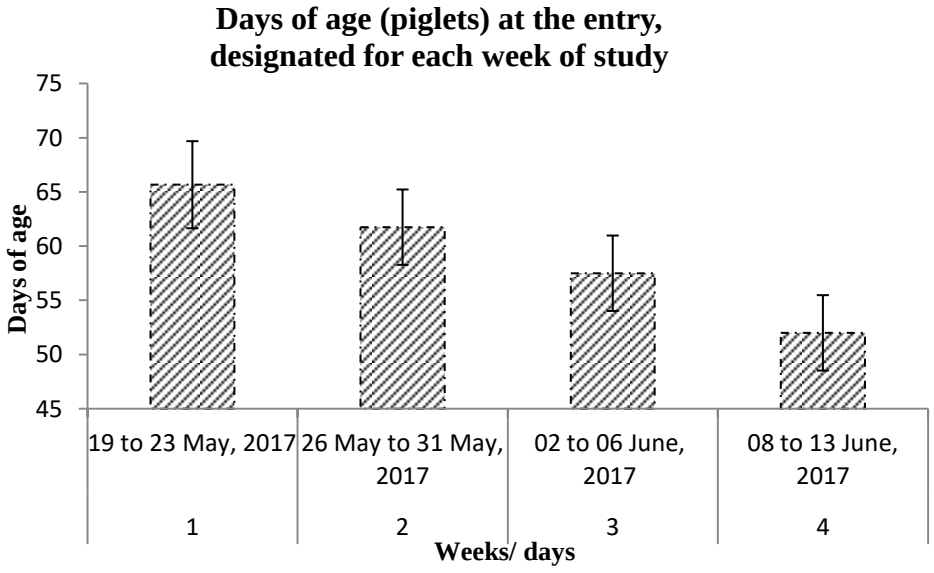

Figure 3 Days of age at the entry

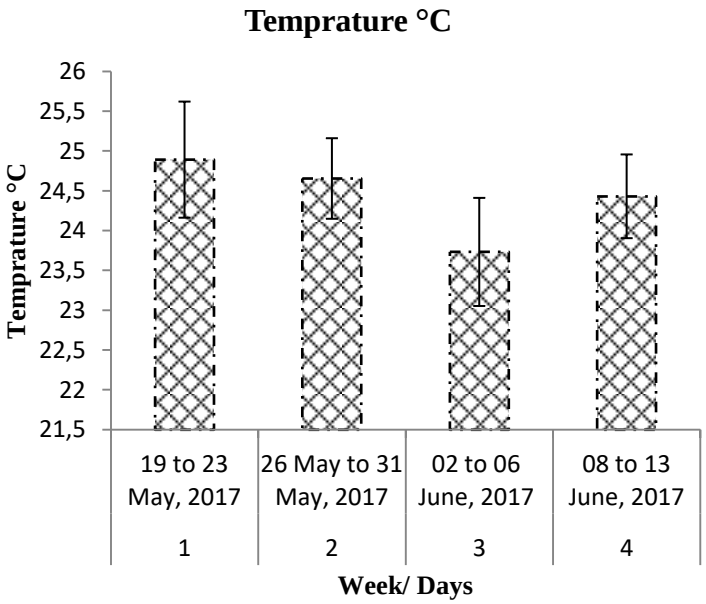

Figure 4 Temperature record

Composition of feed from the origin farm of Duroc pigs

Ingredients	(%)
BM00079- ORDI 9.8 PB	9,224
BM00012- BLAT 10.8	20,000
BM00182- SORGO	5,000
BM00123- COLZA 00 P34	7,000
BM00058- GALETA FARINA Promic	6,000
BM00172- SOJA 46.75	3,136
BM00090- POLPA DE REMOLATXA	3,000
BM00013- MORESC	4,500
BM00071 MELASSA SUGAR PLUS PROTEINA	1,500
BM00019- CARBONAT CÀLCIC	1,050
BM00095- SAL -CLORUR SÒDIC	0,492
BM00427-COR ACABAT BAT E5/02 0.3%	0,300
BM00149- SUP FIT 2000HIPHOS 2.66KGT	0,267
BM00299 TRIPTOFAN 10%EXCIPIENTAT	0,302
BM00002- ACIDS LIQUIDS	0,200
BM00115- TREONINA PURA 985%	0,130
BM77777- AIGUA	0,090
BM00006- ALIMET 88% METIONINA LIQ	0,063
BM00063- BIOLIS 54 % L-Lisina	0,569
BM00024- COLINA CL 75 LIQUIDA	0,040
BM00238- ENZIMS LIQUIDS ROVABIO	0,010
BM00318- GREIX 1º BAT reengrassat	0,787
BM00146- OLI GIRA-SOL ALT OLEIC	0,750
BM00012A- BLAT 13 ALTA	10,000
BM00079A- ORDI 12 ALTA PROT	10,000
BM00057- FOSFAT TRICÀLCIC	0,169
BM00087- PÈSOL	7,828
BM00060- GIRASOL 27.5	2,561
BM00169- SÈGOL 10.5PB	5,000
BM00118- VALINA 20% EXCIPIENTADA	0,034

Chemical composition of feed from the origin farm of Duroc pigs

Nutrients	(%)
Greix Brut%	3,46
Proteïna Bruta%	15,00
Cendres%	4,34
Fibra Bruta%	5,00
Fibra Neutre%	14,29
Sucres (%Glucosa)%	4,49
Midó%	43,27
A.Linoleico C18:2%	1,01
A.Palmitico C16:0%	0,44
A.Estearico C18:0%	0,20
A.Oleico C18:1%	1,50
A.Saturados%	0,75
A.Insaturados%	2,57
A. Mirístic C14:0%	0,02
A. Alfa-linolenic C1%	0,01
P.B.D. porcs%	12,36
A. Palmitoleic C16:1%	0,03
Calci%	0,80
FósforTotal%	0,40
F.dig.porcs%	0,26
Sodi%	0,27
B.eletrolitic%	157,00
Calci anàlisi%	0,67
E-Neta porcs kcalKcal	2285,00
Lisina%	0,94
Lisina/Dig/Porcs%	0,82
Metionina%	0,29
Met/Dig/Porcs%	0,26
Cistina%	0,30
Cist/Dig/Porcs%	0,25
Metionina+Cistina%	0,59
M+C/Dig/Porcs%	0,51
Treonina%	0,64
Treonina/Digs/Porcs%	0,53
Triptofano%	0,19
Triptof/Dig/Porcs%	0,16
Isoleucina%	0,56
Isoleu/Dig/Cerdos%	0,47
Valina%	0,70
Valina/Dig/Porcs%	0,57
Leucina%	1,02
Leu/Dig/Porcs%	0,87

Composition of feed from the origin farm of Pietrain pigs

Ingredients	(%)
BM00012- BLAT 10.8	28,36
BM00079- ORDI 9.8 PB	9,81
BM00172- SOJA 46.75	13,99
BM00013- MORESC	31,49
BM00182- SORGO	7,00
BM00070- GLICERINA GLICEROL 85%	1,20
BM00019- CARBONAT CÀLCIC	1,02
BM00057- FOSFAT TRICÀLCIC	0,50
BM00063- BIOLIS 54 % L-Lisina	0,06
BM00095- SAL -CLORUR SÒDIC	0,50
BM00064- LISINA LIQUIDA 50	0,71
BM00031- CORRECTOR C-1	0,20
BM00002- ACIDS LIQUIDS	0,20
BM00115- TREONINA PURA 985%	0,20
BM00006- ALIMET 88% METIONINA LIQ	0,14
BM77777- AIGUA	0,09
BM00299 TRIPTOFAN 10%EXCIPIENTAT	0,26
BM00238- ENZIMS LIQUIDS ROVABIO	0,01
BM00118- VALINA 20% EXCIPIENTADA	0,20
BM00123- COLZA 00 P34	2,85
BM00062- GREIX ANIMAL 3/5 ACIDESA	1,20
BM00222- FIT L NATUPHOS E 750 0.15	0,02

Chemical composition of feed from the origin farm of Pietrain pigs

Nutrients	(%)
Greix Brut%	3,20
Proteïna Bruta%	15,50
Fibra Bruta%	3,32
Fibra Neutre%	11,05
Sucres (%Glucosa)%	3,87
Midó%	48,00
A.Linoleico C18:2%	1,15
P.B.D. porcs%	13,68
Calci%	0,80
FósforTotal%	0,43
F.dig.porcs%	0,29
Sodi%	0,25
B.eletrolitic%	159,40
Calci anàlisi%	0,66
E-Neta porcs kcalKcal	2391,60
Lisina%	1,06
Lisina/Dig/Porcs%	0,98
Metionina%	0,36
Met/Dig/Porcs%	0,33
Cistina%	0,29
Cist/Dig/Porcs%	0,26
Metionina+Cistina%	0,65
M+C/Dig/Porcs%	0,59
Treonina%	0,73
Treonina/Digs/Porcs%	0,65
Triptofano%	0,20
Triptof/Dig/Porcs%	0,18
Isoleucina%	0,61
Isoleu/Dig/Cerdos%	0,55
Valina%	0,76
Valina/Dig/Porcs%	0,66
Leucina%	1,22
Leu/Dig/Porcs%	1,10